Interaction of β -iodopenicillanate with the β -lactamases of *Streptomyces albus* G and *Actinomadura* R39

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The β -lactamases of *Streptomyces albus* G and *Actinomadura* R39 are inactivated by β -iodopenicillanate. However, in contrast with the β -lactamase I from *Bacillus cereus*, they also efficiently catalyse the hydrolysis of the inactivator; with the *S. albus* G enzyme, k_{cat} is larger than 25 s^{-1} and the number of turnovers before inactivation is 515. With the *A. R39* enzyme, k_{cat} is larger than 50 s^{-1} and the number of turnovers before inactivation is 80. After hydrolysis of the β -lactam amide bond, the product rearranges into 2,3-dihydro-2,2-dimethyl-1,4-thiazine-3,6-dicarboxylate, which exhibits an absorption maximum at 305 nm.

Various β -lactamases are inactivated by 6-halogenopenicillins such as 6-chloropenicillanic acid sulphone (Cartwright & Coulson, 1979), 6- β -bromopenicillanate (Pratt & Loosemore, 1978) and 6- β -iodopenicillanate (Wise *et al.*, 1981; Moore & Brammer, 1981). The interaction between the 6- β -bromo derivative and the β -lactamase I from *Bacillus cereus* has received much attention (Orlek *et al.*, 1979; Knott-Hunziker *et al.*, 1980; Loosemore *et al.*, 1980; Cohen & Pratt, 1980; Orlek *et al.*, 1980). One of the most interesting results was the identification, at the active site of the enzyme, of a serine residue which is irreversibly acylated by β -bromopenicillanate (Knott-Hunziker *et al.*, 1979; Cohen & Pratt, 1980). These results provided the first demonstration that a whole class of β -lactamases are in fact active serine enzymes and that their reaction pathway with penicillins probably involves the formation of an acyl enzyme. Subsequently it was demonstrated that a serine residue is also labelled upon inactivation of the β -lactamase of *S. aureus* by penicillanic acid sulphone (Cartwright & Coulson, 1980) and of that of *Escherichia coli* R TEM by quinacillin sulphone (Fisher *et al.*, 1981). In this latter case, the presence of an acyl enzyme on the reaction pathway with a poor substrate (cefoxitin) was also observed (Fisher *et al.*, 1980a). Finally, a covalent intermediate was trapped at low pH during the hydrolysis of dansyl(5-dimethylamino-naphthalene-1-sulphonyl)-6-aminopenicillanic acid by the β -lactamase I of *B. cereus* (Cartwright & Fink, 1982). These three β -lactamases (*S. aureus*, R TEM and *B. cereus* I) show extensive sequence homology (Ambler, 1979). In all cases, the labelled serine residues are in homologous positions.

The isolation of the β -lactamases from *Streptomyces albus* G and *Actinomadura* R39 has recently been described (Duez *et al.*, 1981, 1982), and indirect experimental evidence has been obtained which suggests that those β -lactamases are serine enzymes (Kelly *et al.*, 1981). Their interaction with β -iodopenicillanate has now been investigated and the results are described herein.

Materials and methods

β -Iodopenicillanic acid (93% purity) was a gift from Pfizer Central Research, Sandwich, Kent, U.K. The β -lactamases of *Streptomyces albus* G and *Actinomadura* R39 were those described by Duez *et al.* (1981, 1982). One unit of β -lactamase hydrolyses 1 μmol of benzylpenicillin/min at 30°C.

The enzyme activities were routinely determined at 30°C and in 50 mM-sodium phosphate buffer, pH 7.0, with 0.1 mM-nitrocefin (a gift of Glaxo Research Laboratories) as substrate (O'Callaghan *et al.*, 1972).

Inactivation experiments were performed at 24°C, in 50 mM-sodium phosphate, pH 7.0, containing, in the case of the *S. albus* G enzyme, 2.5% (v/v) glycerol and 2.5% (v/v) ethylene glycol. For these experiments, a Beckman DU-8 recording spectrophotometer or a u.v./visible Aminco-Morrow DW2 stopped-flow apparatus was used. In the latter case, the optical pathlength of the cuvette was 10 mm.

The enzyme was directly added to a solution containing both nitrocefin and β -iodopenicillanate. In the absence of inactivator, the increase of A_{482} was linear throughout the duration of the experi-

ments. The apparent first-order rate constant of the inactivation reaction was measured by estimating the decrease of the rate of hydrolysis of nitrocefin as described in the preceding paper (Frère *et al.*, 1982b). When the experiments were performed with the Beckman DU-8 spectrophotometer, 5 μ l of enzyme were added to 300 μ l of β -iodopenicillanate and 77 or 90 μ M-nitrocefin. In the 'time-drive' mode, the mixing dead-time did not exceed 7–8 s. When the stopped-flow system was used, one of the syringes delivered about 150 μ l of enzyme solution in phosphate buffer, and the other exactly the same volume of β -iodopenicillanate and 180 μ M-nitrocefin in the same buffer.

Hydrolysis of β -iodopenicillanate

The turnover of β -iodopenicillanate by the *S. albus* G β -lactamase was studied at 24°C in stopped-flow experiments. One syringe delivered about 150 μ l of the β -iodopenicillanate solution in 50 mM-sodium phosphate buffer, pH 7.0, and the other one the same volume of enzyme solution in the same buffer containing 5% (v/v) ethylene glycol and 5% (v/v) glycerol. The final concentration of enzyme was 0.07 μ M, and β -iodopenicillanate concentrations ranged between 15 and 560 μ M. The increase of $A_{305} - A_{325}$ was linear for 1–2 s. The turnover by the *A. R39* β -lactamase was observed at 24°C on the Beckman DU-8 spectrophotometer. Enzyme (0.124 μ M final concn.) was rapidly mixed with concentrations of β -iodopenicillanate ranging from 6.4 to 12.6 μ M. The value of A_{305} was monitored.

Mass spectra

After esterification of the carboxylic functions with diazomethane, amine functions were trifluoroacetylated [15 min at 65°C in a 1:1 (v/v) mixture of trifluoroacetic anhydride and ethyl acetate].

Spectra were obtained with a computerized LKB 2091 spectrometer after gas-chromatographic separation (20 m SE30 capillary column, internal diam. 0.25 mm. The vector gas was helium at a pressure of 152 kPa (1.5 atm). The temperature of the column was increased at 8°C · min⁻¹ (starting at 120°C).

Results

Proposed model

Preliminary experiments indicated that complete inactivation could not be obtained below certain inactivator/enzyme concentration ratios (about 600 for the *S. albus* G enzyme and 100 for the *A. R39* enzyme). When partial enzyme inactivation was observed, the level of residual activity remained stable for 24 h or more. Recovery of activity was

never recorded. On the basis of these results, a simple reaction pathway (model 1) was proposed:

Model 1

In the above model for the interaction between the *A. R39* and *S. albus* G β -lactamases (E) and β -iodopenicillanate (C), EC^* is a common intermediate to both hydrolysis and inactivation pathways (an acyl-enzyme?); EC^I is an irreversibly inactivated complex.

This type of mechanism and conditions which permit individual study of each branch of the pathway are discussed in the preceding paper (Frère *et al.*, 1982b).

Interaction with the *S. albus* G β -lactamase

Complete inactivation reactions. The inactivation reaction was monitored in the presence of 77 μ M nitrocefin at concentrations of β -iodopenicillanate ranging from 9 to 107 μ M. Under these conditions, an apparent first-order inactivation constant $(k_i)_{\text{nit}}$ was determined, the subscript 'nit.' indicating that the value was obtained in the presence of nitrocefin. From this value, K_m and $(k_i)_{\text{lim}}$ could be calculated by using eqn. (2) in the preceding paper (Frère *et al.*, 1982b), which assumed a competition between nitrocefin and β -iodopenicillanate. In the presence of 2.5% ethylene glycol and 2.5% glycerol, the K_m for nitrocefin was 250 μ M (M. Lenzini & J. M. Frère, unpublished work). The values of $(k_i)_{\text{lim}}$ and K_m were obtained by plotting $[C]/(k_i)_{\text{nit}}$ against $[C]$. From this plot, shown in Fig. 1(a), values of 130 μ M and 0.047 s⁻¹, for K_m and $(k_i)_{\text{lim}}$ respectively, were computed (Table 1). The ratio $(k_i)_{\text{lim}}/K_m$ was 360 M⁻¹ · s⁻¹.

Partial inactivation and the k_{+3}/k_{+4} ratio. This ratio was calculated from the residual activity after partial inactivation at low C_0/E_0 ratios. The enzyme (0.178 μ M) was incubated with β -iodopenicillanate concentrations ranging from 0 to 76 μ M. From the residual activity after 30 min at 24°C, a k_{+3}/k_{+4} ratio of 515 ± 40 was obtained (six determinations). During these experiments, the appearance of a compound exhibiting a strong absorbance at 305 nm was observed. The high absorbance values ruled out the possibility of an enzyme-containing complex (EC^I). Assuming that the absorbance was due to product P (or a rearrangement thereof), the measurement of ΔA_{305} during partial inactivation of the enzyme under conditions involving complete turnover of β -iodopenicillanate allowed the computation of a

Fig. 1. Determination of K_m , $(k_i)_{lim}$ and k_{cat} for the interaction of the *S. albus* G β -lactamase with β -iodopenicillanate

(a) Plot of $[C]/(k_i)_{nit.}$ against $[C]$ obtained from complete-inactivation experiments. (b) Plot of $[C]/v$ against $[C]$ for the hydrolysis of β -iodopenicillanate. For conditions, see the text.

molar absorption coefficient of $8150 M^{-1} \cdot cm^{-1}$ for the chromophoric product.

Measurement of product formation. Product formation was monitored at β -iodopenicillanate concentrations ranging from 185 to $558 \mu M$. Experiments were performed with the stopped-flow apparatus. The final enzyme concentration was $69 nM$. Under these conditions, the variation of $A_{305}-A_{325}$ remained linear for about 2 s. A plot of $[C]/v$ against $[C]$ yielded values of $430 \mu M$ and $20 s^{-1}$ for K_m and k_{cat} , respectively (Fig. 1b). Since $k_{cat}/(k_i)_{lim} = 515$, these experiments indicated a value of $0.04 s^{-1}$ for $(k_i)_{lim}$. The K_m obtained in these experiments was thus larger than that obtained in the inactivation experiments. Although this discrepancy in the K_m value could be attributed to experimental errors, the possibility of the presence of two different enzymes in our preparation could not be totally rejected. One of these enzymes would hydrolyse nitrocefin and be inactivated by β -iodopenicillanate, whereas the second one would only metabolize the latter β -lactam. To eliminate this possibility, the following experiment was performed: to $400 \mu l$ of $0.178 \mu M$ -enzyme, $10 \mu l$ portions of $875 \mu M$ - β -iodopenicillanate were successively added. After each addition, the mixture was incubated for 10 min at $24^\circ C$. The A_{305} was then determined and a $2 \mu l$ sample was added to $350 \mu l$ of $100 \mu M$ -nitrocefin. After four additions ($C_0/E_0 = 480$), the enzyme was completely inactivated (as judged by incubation with nitrocefin), and a further addition of β -iodopenicillanate did not produce any additional increase of A_{305} . Thus the amount of β -iodopenicillanate necessary to induce the disappearance of the nitrocefin-hydrolysing activity also eliminated the turnover of β -iodopenicillanate itself. In consequence, both activities were probably due to the same enzyme.

Table 1. Values of the constants involved in the interactions between β -iodopenicillanate and the *S. albus* G and *A. R39* β -lactamases

$(k_i)_{lim}$ characterizes the inactivation rate at saturating concentrations of β -iodopenicillanate. For the *S. albus* G β -lactamase, the different K_m values are responsible for corresponding differences in the $(k_i)_{lim}/K_m$ and k_{cat}/K_m values.

	<i>S. albus</i> G lactamase	<i>A. R39</i> lactamase	Method
1. k_{+3}/k_{+4}	515 ± 40	80 ± 13	Partial inactivation
2. $(k_i)_{lim}$	$0.05 s^{-1}$	$> 0.3 s^{-1}$	Complete inactivation
	$0.04 s^{-1}$	$> 0.64 s^{-1}$	Initial rate of β -iodopenicillanate turnover and 1. above
3. K_m	$130 \mu M$	$> 33 \mu M$	Complete inactivation
	$400 \mu M$	$> 40 \mu M$	Initial rate of β -iodopenicillanate turnover
4. k_{cat}	$25 s^{-1}$	$> 24 s^{-1}$	Complete inactivation and 1. above
	$20 s^{-1}$	$> 50 s^{-1}$	Initial rate of β -iodopenicillanate turnover
5. $\frac{(k_i)_{lim}}{K_m} = \frac{k_{+4}}{k_{+3}} \frac{k_{cat}}{K_m}$	$360 M^{-1} \cdot s^{-1}$	$16000 M^{-1} \cdot s^{-1}$	Complete inactivation
	$100 M^{-1} \cdot s^{-1}$	$> 10000 M^{-1} \cdot s^{-1}$	Initial rate of β -iodopenicillanate turnover
6. $\frac{k_{+2}}{K} = \frac{k_{cat}}{K_m}$	$185000 M^{-1} \cdot s^{-1}$	$1.3 \times 10^6 M^{-1} \cdot s^{-1}$	Complete inactivation and 1. above
	$50000 M^{-1} \cdot s^{-1}$	$> 0.8 \times 10^6 M^{-1} \cdot s^{-1}$	Turnover of β -iodopenicillanate

Interaction with the *A. R39* β -lactamase

Partial inactivation. The enzyme ($0.013\ \mu\text{M}$) was incubated with β -iodopenicillanate concentrations ranging from 0.275 to $2.2\ \mu\text{M}$ (C_0/E_0 ratios from 21 to 167). For C_0/E_0 ratios higher than 85, no residual activity was observed after 15 min. From the stable residual activity observed at lower C_0/E_0 ratios, a value of 80 ± 13 (ten determinations) was observed for $k_{\text{cat.}}/(k_i)_{\text{lim}}$.

Complete inactivation experiments. The rate of inactivation was measured by using the continuous procedure in the presence of nitrocefin. Two series of experiments were carried out with the help of the Beckman DU-8 and the stopped-flow apparatus respectively. With β -iodopenicillanate concentrations ranging from 0.94 to $33\ \mu\text{M}$, apparent inactivation rate constants ranging from 7.5×10^{-3} to $0.292\ \text{s}^{-1}$ were obtained, strictly proportional to the inactivator concentrations. Further studies, with higher concentrations of β -iodopenicillanate, would have required the utilization of too large amounts of enzyme and were not attempted. An average value of $8100 \pm 900\ \text{M}^{-1}\cdot\text{s}^{-1}$ was found for the ratio $(k_i)_{\text{nit.}}/[C]$. From eqn. (2) in the preceding paper (Frère *et al.*, 1982b), it can be seen that if $[C] \ll K_{m_i}$:

$$\frac{(k_i)_{\text{lim}}}{K_{m_i}} = \frac{(k_i)_{\text{nit.}}}{[C]} \left(1 + \frac{[S]}{K_{m_2}} \right)$$

where $[C]$ and K_{m_i} are the concentration of, and the K_m for, β -iodopenicillanate, and $[S]$ and K_{m_2} the concentration of, and the K_m for, nitrocefin respectively. The value of

$$\frac{(k_i)_{\text{lim}}}{K_{m_i}} = \frac{k_{+4}}{k_{+3}} \cdot \frac{k_{+2}}{K}$$

was thus $16\,000 \pm 1800\ \text{M}^{-1}\cdot\text{s}^{-1}$.

Measurement of product formation. When $0.124\ \mu\text{M}$ -enzyme was mixed with β -iodopenicillanate concentrations ranging from 6.4 to $12.6\ \mu\text{M}$, an increase in A_{305} was observed. Again, this increase was too high to result from the formation of a complex containing the enzyme, and could only be due to the product of β -iodopenicillanate turnover. Since the experiments had been carried out with the DU-8 spectrophotometer, the first 50% of the increase of absorbance could not be monitored, owing to the 8 s mixing dead-time. The available amount of enzyme was not sufficient to perform stopped-flow experiments, but a *minimum* value of k_{+2}/K could be calculated from these results. In absence of enzyme inactivation, the reaction should have been first-order, since the β -iodopenicillanate concentrations used were well below the K_m value. However, inactivation and turnover did occur simultaneously, and the amount of product observed after 8 s was in fact somewhat lower than that

expected from a simple first-order process. Thus a calculation based on the first observed absorbances after the mixing dead-time supplied a minimum value for the real first-order rate constant ($k_{+2}E_0/K$). On this basis, k_{+2}/K was greater than $8 \times 10^5\ \text{M}^{-1}\cdot\text{s}^{-1}$.

Table 1 summarizes the values of the various constants observed or calculated for each enzyme.

Identification of the product

The presence of a maximum of absorbance at 305 nm suggested that the product of β -iodopenicillanate turnover could be the 2,3-dihydro-2,2-dimethyl-1,4-thiazine-3,6-dicarboxylate (compound A):

which is also formed by incubation of β -bromopenicillanate in the presence of OH^- ions (Cohen & Pratt, 1980). A standard was prepared by incubating β -iodopenicillanate for 30 min at 37°C in 1 M-potassium phosphate, pH 12. Appearance of the chromophore at 305 nm obeyed first-order kinetics, which indicated that the rearrangement into compound (A) very rapidly followed the hydrolysis of the β -lactam amide bond. The pseudo-first-order rate constant was $1 \times 10^{-3}\ \text{s}^{-1}$, a value in the range of those observed for the OH^- -catalysed hydrolysis of various penicillins (Frère *et al.*, 1982a).

Spectra of the product were recorded at pH 12.0 and 7.0. These spectra were superimposable and the molar absorption coefficient was $8200\ \text{M}^{-1}\cdot\text{cm}^{-1}$, in excellent agreement with the value published by Cohen & Pratt (1980). A shoulder was also observed between 260 and 270 nm, with $\epsilon_{265} = 0.55 - 0.65\epsilon_{305}$. At pH 7.0, however, the compound was unstable, and the chromophore slowly disappeared with half-lives of 169 and 44 min at 20° and 37°C respectively. U.v. spectra of the products formed during the interaction with the enzymes were obtained as follows. The *S. albus* G β -lactamase ($0.12\ \mu\text{M}$) was incubated with $19\ \mu\text{M}$ and the *A. R39* β -lactamase ($0.13\ \mu\text{M}$) with $5.95\ \mu\text{M}$ - β -iodopenicillanate. After 10 min at 24°C , A_{305} reached a maximum and the spectra were recorded. They were superimposable on that of the product obtained by chemical hydrolysis of β -iodopenicillanate at high pH. The calculated molar absorption coefficients were 8200 and 6900 for the products of turnover by the *S. albus* G and *A. R39* β -lactamases respectively. The shoulder between 260 and 270 nm was also present in both cases.

Identification of the product obtained by interaction with the *S. albus* G enzyme was performed

Fig. 2. Mass spectra of the standard 2,3-dihydro-2,2-dimethyl-1,4-thiazine-3,6-dicarboxylate (a) and of the product obtained by interaction of the *S. albus* G β -lactamase with β -iodopenicillanate (b) after esterification and trifluoroacetylation

The molecular ion ($M^+ = 341$) is the most intense. Other m/e ratios are: 326 ($M^+ - \text{CH}_3$), 310 ($M^+ - \text{OCH}_3$), 294 ($M^+ - \text{CH}_3 - \text{OCH}_3 - \text{H}$), 282 ($M^+ - \text{CO}_2\text{CH}_3$), 250 ($M^+ - \text{OCH}_3 - \text{CO}_2\text{CH}_3 - \text{H}$), 224 ($M^+ - \text{COCF}_3$), 240 ($M^+ - \text{OCH}_3 - \text{COCF}_3 - \text{H}$?), 223 ($M^+ - 2 \times \text{CO}_2\text{CH}_3$), 212 ($240 - \text{CO}$), 185 ($M^+ - \text{COCF}_3 - \text{CO}_2\text{CH}_3$), 170 ($185 - \text{CH}_3$).

by mass spectrometry. In this case, the standard was prepared by dissolving 2.6 mg of β -iodopenicillanate in 0.8 ml of 25 mM-NaOH and incubating the mixture at 37°C. In turn, 60 nmol of β -iodo-

penicillanate were incubated for 10 min at 24°C in the presence of 0.1 nmol of *S. albus* G β -lactamase in 500 μl of 50 mM-sodium phosphate, pH 7.0. After 15 min, the solutions were cooled to 0°C, added to

1 ml of 1M-potassium phosphate, pH 1.85, and extracted three times with 1.5 ml of diethyl ether. After redissolution of a dry-evaporated sample of the diethyl ether phase of the standard in 50mm-phosphate, pH 7.0, the characteristic u.v. spectrum of compound (A) was observed. To the dry-evaporated residues, a solution of diazomethane in ether was added. The mass spectra (not shown) were identical. The principal m/e ratios were 245 (M^+), 214 ($M^+ - OCH_3$), 186 ($M^+ - CO_2CH_3$), 171 ($M^+ - CH_3 - CO_2CH_3$), 154 ($M^+ - OCH_3 - CO_2CH_3 - H$), 126 ($M^+ - 2 \times CO_2CH_3 - H$).

After trifluoroacetylation, identical mass spectra were again obtained (Fig. 2). These data clearly established that the compound formed upon interaction with the *S. albus* G enzyme was the dihydrothiazine, compound (A). On the basis of the identical u.v. spectra, it was highly probable that the interaction with the *A. R39* enzyme yielded the same product.

Discussion

The present results show for the first time that turnover of β -iodopenicillanate by a β -lactamase can occur simultaneously with enzyme inactivation. The

measured value of the K_m was, for the *S. albus* G β -lactamase, somewhat different (by a factor of 3), depending upon the phenomenon which was monitored. This difference might be due in part to experimental error, and in part to the simplifications introduced to make the kinetics more amenable to analysis. However, the separate studies of both pathways yielded values which were generally in good agreement.

If the values of k_{cat} and K_m obtained for β -iodopenicillanate and clavulanate (the preceding paper, Frère *et al.*, 1982b) are compared with those obtained for the usual substrates of these β -lactamases (Duez *et al.*, 1981; Frère *et al.*, 1982a), β -iodopenicillanate and clavulanate can be considered as good substrates. Specifically, the values of k_{+2}/K (k_{cat}/K_m) are close to those observed for the best substrates. However, β -iodopenicillanate and clavulanate also inactivate both enzymes and, in both cases, β -iodopenicillanate is a better inactivator.

On the basis of our results and of the observation of an enzyme-bound chromophore by Loosemore *et al.* (1980), an interaction scheme starting with the acyl-enzyme, I (Scheme 1) can be proposed.

The acyl-enzyme I is probably the common

Scheme 1. Possible pathway for the reaction between the β -lactamases and β -iodopenicillanate

Scheme 2. Alternative pathway for the formation of the dihydrothiazine (VI)

intermediate to both pathways, since the ester bond would be expected to be much more stable in (II) and (III) than in (I) (Scheme 1) (Charnas & Knowles, 1981; Cohen & Pratt, 1980). The involvement of a base (B_2), which would abstract the 6- α proton has been proposed by Fisher *et al.* (1980b). Whether this base is part of the enzyme and the same as the one (B_1^*) that would be involved in the transformation of IV to V is impossible to decide at this stage. An alternative proposal (Scheme 1 in Cohen & Pratt, 1980) does not require the presence of a base, but also results in the formation of a dihydrothiazine attached to the enzyme active serine residue. The rearrangement of IV into VI could follow a similar pathway (Scheme 2).

Although the hydrolysis and inactivation pathways need not necessarily be symmetrical, our identification of the dihydrothiazine (compound A) as the product of the reaction brings further support to the assumption by Cohen & Pratt (1980) that the enzyme-bound chromophore cannot be a tetrahydrothiazepine. It is important to note, finally, that with the two enzymes under study in the present paper, a small C-6 side chain does not protect a substrate against hydrolysis. This had already been noticed previously with penicillanate (Frère *et al.*, 1982a). On the contrary, both enzymes hydrolyse β -iodopenicillanate with a high efficiency before they

are inactivated by a mechanism-based reaction. Branched pathways similar to those discussed here and by Fisher *et al.* (1980b) might be rather general phenomena in the interactions between β -lactamases and mechanism-based reagents.

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References

- Ambler, R. P. (1979) in *β -Lactamases* (Hamilton-Miller, J. M. T., ed.), pp. 99–125, Academic Press, London and New York
- Cartwright, S. J. & Coulson, A. F. W. (1979) *Nature (London)* **278**, 360–361
- Cartwright, S. J. & Coulson, A. F. W. (1980) *Philos. Trans. R. Soc. London Ser. B* **289**, 370–372
- Cartwright, S. J. & Fink, A. L. (1982) *FEBS Lett.* **132**, 186–188
- Charnas, R. L. & Knowles, J. R. (1981) *Biochemistry* **20**, 3214–3219

- Cohen, S. A. & Pratt, R. F. (1980) *Biochemistry* **19**, 3996–4003
- Duez, C., Frère, J. M., Klein, D., Noël, M., Ghuysen, J. M., Delcambe, L. & Dierickx, L. (1981) *Biochem. J.* **193**, 75–82
- Duez, C., Frère, J. M., Ghuysen, J. M., Van Beeumen, J., Delcambe, L. & Dierickx, L. (1982) *Biochim. Biophys. Acta* **700**, 24–32
- Fisher, J., Belasco, J., Koshla, S. & Knowles, J. R. (1980a) *Biochemistry* **19**, 2895–2901
- Fisher, J., Belasco, J., Charnas, R. L., Koshla, S. & Knowles, J. R. (1980b) *Philos. Trans. R. Soc. London Ser. B* **289**, 309–319
- Fisher, J., Charnas, R. L. & Knowles, J. R. (1981) *Biochemistry* **20**, 2726–2731
- Frère, J. M., Kelly, J. A., Klein, D., Ghuysen, J. M., Claes, P. & Vanderhaeghe, H. (1982a) *Biochem. J.* **203**, 223–234
- Frère, J. M., Dormans, C., Lenzini, V. M. & Duyckaerts, C. (1982b) *Biochem. J.* **207**, 429–436
- Kelly, J. A., Frère, J. M., Duez, C. & Ghuysen, J. M. (1981) *Biochem. J.* **199**, 137–143
- Knott-Hunziker, V., Waley, S. G., Orlek, B. S. & Sammes, P. G. (1979) *FEBS Lett.* **99**, 59–61
- Knott-Hunziker, V., Orlek, B. S., Sammes, P. G. & Waley, S. G. (1980) *Biochem. J.* **187**, 797–802
- Loosemore, M. J., Cohen, S. A. & Pratt, R. (1980) *Biochemistry* **19**, 3990–3995
- Moore, B. A. & Brammer, K. W. (1981) *Antimicrob. Agents Chemother.* **20**, 327–331
- O'Callaghan, C., Morris, A., Kirby, S. A. & Shingler, A. H. (1972) *Antimicrob. Agents Chemother.* **1**, 283–288
- Orlek, B. S., Sammes, P. G., Knott-Hunziker, V. & Waley, S. G. (1979) *J. Chem. Soc. Chem. Commun.* 962–963
- Orlek, B. S., Sammes, P. G., Knott-Hunziker, V. & Waley, S. G. (1980) *J. Chem. Soc. Perkin Trans. I*, 2322–2329
- Pratt, F. & Loosemore, M. J. (1978) *Proc. Natl. Acad. Sci. U.S.A.* **75**, 4145–4149
- Wise, R., Andrews, J. M. & Patel, N. (1981) *J. Antimicrob. Agents Chemother.* **7**, 531–536